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Cytotoxicity against Hela (Cervical Cancer) Cell Line Proliferation and MCF7 (Breast Cancer) Cell Line of Combretum Adenogonium

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Abstract

The importance of Sudanese medicinal plants, were shown to inhibit the growth of human cancer cell lines originated from Hela (Cervical Cancer) and Anti-Oxidants activity (DPPH). This work investigated the anticancer, antioxidant and Cytotoxicity activities of *C. adenogonium* roots, leaves, and stems commonly used as anti-inflammatory and anti-tumor. All the plant parts were extracted using 80% methanol, the anticancer activity was examined by using MTT assay against Hela (Cervical Cancer) and MCF7 (Breast Cancer) Cell Line. And determine their antioxidant activities by testing DPPH cytotoxicity using - (4, 5-Dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), filter and kept in dark, prepared freshly. All the examined plants methanolic extracts are very high activity which are *C. adenogonium* stem, *C. adenogonium* leaves, *C. adenogonium* roots, their IC_{50} are 1.03, 5.4, 7.2, µg/ml respectively. The extract *C. adenogonium* leaves DC has shown very high activity against Hela (Cervical Cancer) and roots, and stems has shown none active anti- Hela (Cervical Cancer) IC_{50} values 43.8, > 100, and > 100 µg/ml respectively. All the extracts revealed cytotoxicity activity against MCF7 (Breast Cancer) Cell Line has shown none active in all plants parts under study *C. adenogonium* roots, leaves, and stems. All the extracts revealed cytotoxicity activity against not toxic in roots, leaves and stems the inhibition percentage with (86.4, 86.4, 84.5) (88.7, 82. 4, 75.8) (83.6, 82.1, 54.7) respectively.

Keywords: *PC3; Chemiluminescence; Anticancer; Medicinal plants; Cytotoxicity*

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Introduction

Cancer is a complex disease and it is impossible to give a full account of its occurrence, prevention and treatment, however, it can be characterized by abnormal cell proliferation in which cell growth is uncontrolled, cells develop abnormal sizes and shapes, destroy their adjacent tissues, and can ultimately spread to other organs and tissues. Multiple factors are involved in the etiology of cancer such as ionizing radiations, tobacco, cigarette smoke, betel nut, unhealthy life style and carcinogens present in the workplace (naphthylamines and coal tar derivatives). The most frequently observed genetic lesion in human cancer is the mutation of the p53 tumor suppressor gene

(Levine, 1997). It is well established that chronic infections by the hepatitis B and C viruses, human papillomaviruses and *Helicobacter pylori* lead to the cancers of liver, cervix and stomach, respectively (Monto and Wright, 2001, Uemura., *et al*. 2001, Hausen, 2002, Suerbaum and Michetti, 2002). Additionally, mycotoxins appear to be strong liver High incidence of oral cancer has been reported to be associated with betel quid chewing (Shirname., *et al*. 1983, Trivedy., *et al*. 2002).

Cancer development can be prevented by surgical removal (pre-malignant lesions as in case of cervical dysplastic lesions and colon adenomas), vaccines (against human papillomavirus and hepatitis B virus), and prevention of food crop contamination (aflatoxin) (Fong., *et al*. 2001). On the contrary people consuming food rich in fruits and vegetables have a lower incidence of various cancers and this has been mainly related to their antioxidant (β-carotene) properties (Baron., *et al*. 2003).

Cancer is a leading cause of death worldwide, accounting for 7.6 million deaths (around 13% of all deaths) in 2008.Lung, stomach, liver, colon and breast cancer cause the most causes of cancer deaths each year, the most frequent types of cancer differ between men and women, About 30% of cancer deaths are due to the five leading behavioral and dietary risks: high body mass index, low fruit and vegetable intake, lack of physical activity, tobacco use, alcohol use, tobacco use is the most important risk factor for cancer causing 22% of global cancer deaths and 71% of global lung cancer deaths. Cancer causing viral infections such as HBV/HCV and HPV are responsible for up to 20% of cancer deaths in low- and middle-income countries, about 70% of all cancer deaths in 2008 occurred in low- and middle-income countries deaths from cancer worldwide are projected to continue rising, with an estimated 13.1 million deaths in 2030 (WHO. 2012).

Aims of the study

1. To evaluate anticancer activities residing in the plants extracts and/or fractions using human cancer cell lines (MCF7, Hela).
2. To select indigenous plant from Sudanese flora suggested as anti-cancer depending on folk medicine uses.
3. To investigate antioxidant activity (DPPH and metal chelating) for the selected plant parts
4. To investigate the cytotoxicity of the selected plant parts.

Material and Methods

Collection of tested plant parts of the *C. adenogonium* Collected from the Farm of Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan (MAPRI) during the period of June and July 2010 and identified of taxonomist team of Medicinal and Aromatic Plants Research Institute, National Center of Research, Khartoum, Sudan. And herbarium voucher was deposited at herbarium medicinal plants in the MAPRI.

Preparation of the Crude Extracts

The plant parts were shade dried. The powdered materials were accurately weighed and extracted by shakerSF_1 from (Stuart Scientific, made in Great Britain). Of room temperature by using 80% methanol. After that the filtered extracts were concentrated on rotatory BUCHI 461 evaporator. The extracts, were dried and concentrated by using hood extracts and the yield percentage was then calculated.

Fractionation by Solvent Solvent System

Specific weight of each sample methanol extract was dissolved in 250 ml distilled water and transferred to 500 ml capacity separating funnel. 100 ml of ethyl acetate was added, shacked gently and allowed to stand till two layers appeared clear. Ethyl acetate layer separated in conical flask and the aqueous was shacked two times more with 100 ml of ethyl acetate in each time. Ethyl acetate layers combined together and evaporated under reduced pressure using rotary evaporator. Aqueous layer was lyophilized using freez dryer apparatus (Freeze drier from (TMVAC made in USA) and the yield percentages of both fractions was calculated (Harborne, 1984).

Extraction and Phytochemical Screening

General phytochemical screening to detect the chemical groups was carried out for all extracts using the methods described by (Sukhdev., *et al*. 2008; Martinez& Valencia, 1999; Sofowora, 1993; Harborne, 1984; and Wall., *et al*. 1952) with few modifications.

Identification of Tannins

0.2g of each extract was dissolved in 10 ml of hot saline solution and divided in two test tubes. In the first tube 2-3 drops of ferric chloride were added and 2-3 drops of gelatin salt reagent were added to the other tube. The occurrence of a blackish blue colour in the first test tube and turbidity in the second one indicates the presence of tannins.

Test of Sterols and Triterpenes

0.2g of each extract was dissolved in 10 ml of chloroform, to 50ml of the solution 0.5 ml acetic anhydride was added and then 3 drops of conc. Sulphuric acid at the bottom of the test tube. At the contact zone of the two liquids a gradual appearance of green, blue pink to purple color is indicates the presence of sterols (green to blue) and or triterpenses (pink to purple) in the sample.

Test for Alkaloids

0.5 g of each extract was dissolved in 2 ml of 2N HCl in water bath and stirred while heating for 10 minutes, then cooled filtered and divided into two test tubes. To the first test tube a few drops of Mayer's reagent were added while to the other tube a few drops of Valser's reagent were added. A slight turbidity or heavy precipitate in either of the two test tubes indicates the presence of alkaloids.

Tests for Flavonoids

0.5g of each extract was dissolved in 30 ml of 80% ethanol and filtered.

The filtrate was used for following tests: -

- A/ to 3 ml of the filtrate in a test tube 1 ml of 1% aluminum chloride solution was in methanol was added. Formation of a yellow color indicated the presence of Flavonoids. Flavones and or chalcone.
- B/ to 3 ml of the filtrate in a test tube 1 ml of 1% potassium hydroxide solution was added. A dark yellow color indicated the presence of Flavonoids compounds (flavones or flavonones) chalcone and/or flavonols.
- C/ to 2 ml of the filtrate 0.5ml of magnesium turnings were added. Production of a faint pink or red color was taken as presumptive evidence that flavonones are present in the plant sample.

Test for Saponins

0.3 g of each extract was placed in a clean test tube. 10 ml of distilled water were added, the tube stoppered and vigorously shaken for about 30 seconds. The tube was then allowed to stand and observed for the formation of foam, which persisted for at least one hour, was taken as evidence for presence of saponins.

Test for Cumarins

0.2g of each extract dissolved in 10 ml distilled water in test tube and filter paper attached to the test tube to be saturated with the vapor after a spot of 0.5N KoH put on it. Then the filter paper was inspected under UV light, the presence of coumrins was indicated if the spot was found to have adsorbed the UV light.

Test for Anthraquinone glycosides

0.2g of each extract was boiled with 10 ml of 0.5N KoH containing 1 ml of 3% hydrogen peroxide solution. The mixture was extracted by shaking with 10 ml of benzene. 5ml of the benzene solution was shacken with 3 ml of 10% ammonium hydroxide solution and the two layers were allowed to separate. The presence of anthraquinones was indicated if the alkaline layer was found to have assumed pink or red color.

Test for Cyanogenic glycosides

0.2g of extract fraction was placed in Erlenmeyer flask and sufficient amount of water was added to moisten the sample, followed by 1ml of chloroform (to enhance every activity). A piece of freshly prepared sodium picrate paper was carefully inserted between a split cork which was used to stopper the flask, a change in color of the sodium picrate paper from yellow to various shades of red was taken as an indication of the presence of cyanogenic glycoside.

Antioxidant assay

DPPH free radical scavenging activity method

A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods. It has also been used to quantify antioxidants in complex biological systems in recent years.

The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to number of electrons captured.

Procedure

The DPPH radical scavenging was determined according to the method of Shimada., *et al*. (1992), with whose modification. In 96-wells plate, the test samples were allowed to react with 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37ºC. The concentration of DPPH was kept as (300µM). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

Percent of DPPH inhibition= [(AB-AA)/AB] × 100

Where; AA and AB are the absorbance values of the test and of the blank sample, respectively. A percent inhibition versus concentration curve was plotted.

Culture media and human tumor cell lines

Human Cell lines

PC3 (prostate cancer cell line) were obtained frozen in liquid nitrogen (-180°C), the tumor cell lines were maintained in the Institute of ICCB, University of Karachi Pakistan.

Culture Media

RPMI -1640 medium was used for culturing and maintenance of the human tumor cell lines. The medium was supplied in a soluble form. Before using the medium it was warm at 37°C in a water bath and supplemented with penicillin/streptomycin and Fetal bovine serum (FBS) with 10% concentration. The cells were maintained at 37°C in a humidified atmosphere with 5% CO_2 and were sub cultured twice a week.

Procedure

Maintenance of the human cancer cell lines in the laboratory

A cryo tube containing frozen cells was taken out of the liquid nitrogen container and then thawed in a water bath at 37oC. The cryo tube was opened under strict aseptic conditions and its content was supplied by 5 ml complete media (RPMI- 1640 with 10%

fetal bovine serum) drop by drop in a 50 ml disposable sterile falcon tubes and were centrifuged at 1200 rpm for 10 min to discard the preserving solution. The supernatant was discarded and the cell pellet was seeded in 5 ml complete media in T25 Nunclon sterile tissue culture flasks. The cell suspension was incubated at 37°C in a humidified atmosphere with 5% CO_2 and followed up daily with changing the supplemented medium every 2-3 days. Incubation was continued until a confluent growth was achieved and the cells were freshly sub cultured before each experiment.

Collection of Cells by Trypsinization

The media was discarded. The cell monolayer was washed twice with 5 ml phosphate buffered saline and all the adherent cells were dispersed from their monolayer by the addition of 1 ml trypsin solution (0.025% trypsin w/v) for 2 minutes. The flask was left in the incubator till complete detachment of all the cells and checked with the inverted microscope (Olympus). Trypsin was inactivated by the addition of 5 ml of the complete media. The trypsin content was discarded by centrifugation at 1200 rpm for 10 minutes. The supernatant was discarded and the cells were separated into single cell suspension by gentle dispersion several times, then suspended and seeded in 5 ml complete media in T25 Nunclon sterile tissue culture flasks.

Determination and counting of viable cells

50 µl of fresh culture media was added to 50 µl of the single cell suspension. The cells were examined under the inverted microscope using the haemocytometer. Viable cells were counted and the following equation was used to calculate the cell count/ml of cell suspension.

$$\text{Viable cells/ml} = \frac{\text{number of cells in 4 quarters} \times 2 \text{ (dilution factor)} \times 10^4}{4}$$

The cells were then diluted to give the concentration of single cell suspension required for each experiment. The cell count was adjusted to 1 x 10^4 -10^5 cells/ml using medium containing 10% fetal bovine serum.

Cryopreservation of cells

To avoid the loss of the cell line, excess cells were preserved in liquid nitrogen as follows: Equal parts of the cell suspension and freezing medium (10% DMSO in complete media) were dispersed to cryo tubes. The cryo tubes were racked in appropriately labeled polystyrene boxes gradually cooled till reaching -80°C. Then the cry tubes were transferred to a liquid nitrogen (-196°C).

Micro Culture Tetrazolium (Mtt) Assay

MTT assay

In order to evaluate the cytotoxicity effect of the extracts and compounds, the following procedure of the MTT was used.

MTT procedure

Serial dilutions of extract were prepared in a 96 well flat bottomed plate (Nalge Nunc, Inter.). The outer wells of the plate were filled with 250 µl of in-complete culture medium except the last row 6 middle wells (B - G), which were used for the negative control receiving 50 µl of culture medium and 2µl of sterile 0.5% Triton x.

To the rest of the plate, 50 µl/wells (CCM) were added and 30 µl more were added to second column wells (B – G) that were used as first extract dilution wells. To the first dilution wells in the row, 500 µg of c suspension extract were added to the 80 µl. extract were then serially diluted by two-fold dilution from well B3 till B11 by transferring 250 µl to the next well after proper mixing. From the last dilution wells (B-11), 50 µl were discarded. Each compound was tested in triplicate. Cell suspension in a complete culture medium containing 2.5 X 10^5/ml was properly mixed, and 150 µl of it were transferred into each well of the plate. The plate was covered and placed in 5% CO_2 incubator at 37°C for three-five days (72 hours-120 hours). On the third/fifth day, the supernatant was removed from

each well without detaching cells. MTT stock (5 mg/ml) was prepared earlier in 100 ml PBS. MTT suspension was vortexed and kept on magnetic stirrer until all MTT dissolved. The clear suspension was filtered sterilized with 0.2 µ Millipore filter and stored at 4°C or -20 until use. MTT was diluted (1:3.5) in a culture medium and brought to room temperature. To each well of the 96 well plates, 50 µl of diluted MTT were added. The plate was incubated further at 37°C for 2 to 3 hours in CO_2 incubator. MTT was removed carefully without detaching cells, and 200 µl of DMSO were added to each well. The plate was agitated at room temperature for 15 minutes then read at 540 nm using micro plate reader. %Inhibition = [(A Control – A Sample)/A Control] × 100

Where A Control is the absorbance of the negative control and A Sample the absorbance of tested samples or standard. All data are an average of triplicate analyses.

Statistical Analysis

All data are presented as mean ± standard deviation of the mean - statistical analysis for all the assays result were done using students t-test significance was tribute to probability values $P < 0.05$ or $P < 0.01$ in some cases.

Result and Discussion

Sudan is one of the developing countries that face a great challenge with cancer management. About 5700 cases had been seen during year 2007 in Radiation and Isotope Center- Khartoum (RICK) and Institute of Nuclear medicine, Molecular biology, and Oncology (INMO) Wadmedani which are the only cancer centers in Sudan (Dafalla., *et al*. 2007).

Medicinal plants are plants that have at least one of their parts (leaves, stem, barks or roots) used for therapeutic purposes (Bruneton, 1993). Recently, medicinal plants have become important for the treatment of different disease conditions, such as diabetes, malaria, anaemia (Fola, 1993). The availability and relatively cheaper cost of medicinal plants in sub-Saharan Africa, makes them more attractive as therapeutic agents when compared to 'modern' medicines (Agbor and Ngogang, 2005; Agbor., *et al*. 2005).

In the family Combretaceae, the anticancer activity for the above assay of methanolic extracts of *C. adenogonium* roots, *C. adenogonium* leaves, and activity against Hella cell lines was $IC_{50} > 100$, 43.85, µg/ml respectively. Very high activity against Hella cell lines (IC50 43.85 µg/ml). *C. adenogonium* leaves were found to be not toxic on normal cell lines (IC_{50} 8.5 µg/ml). Phytochemically, leaves extract indicated the presence of cumarins, saponins in trace amount, and triterpenes in moderate amount, tannins and flavonoids in high amount, alkaloids, sterols and anthraquenones were not detected. A previous study on the biological activity two extracts, namely, stem bark and leaf extracts showed mild toxicity with LC_{50} values of 65.768 µg/ml and 76.965 µg/ml, respectively, whereas roots were relatively non-toxic LC_{50} 110.042 µg/ml, anti-HIV-1 protease activity, toxicity properties roots and stem bark exhibited anti-HIV-1 PR activity with IC_{50} values of 24.7 and 26.5 µg/ml, respectively (Mushi., *et al*. 2012).and phytochemicals in extracts from *C. adenogonium* to evaluate potential of these extracts for development as herbal remedies Phytochemical screening of the extracts indicated presence of flavonoids, terpenoids, alkaloids, tannins, glycosides and saponins (Mushi., *et al*. 2012).

Also the previous study in biological activity the plants bark, leaves and roots were extracted for traditional medicinal uses for treating various ailments from influenza, and rheumatism, to sexual issues such as impotence and syphilis. It is commonly brewed as a tea in tropical West Africa to relieve stomach issues, and to treat malaria in a decoction with a number of other leaves obtained in the bush. In the Senegambia region, it is one of a number of trees whose twigs are used as "chewing sticks," used in lieu of tooth brushes to clean teeth, remove food particles after eating, and to chew for pleasure. It is also used to fill cavities of carious teeth, to dress wounds, for fumigation and as incense. A decoction of the leaves is used for baths to relieve fatigue. Chemical analysis of the leaves of the plant has identified Galic acid, ellegic acid, flavonoid glycoside and 4 tannins; the tannins reported are 2, 3 - (S)-hexahydr oxydiphenol-D-glucose, punicalin, punicalagin and combreglutinin. The black colour of the bogolan fabric is attributed to the chemical reaction of the tannins with the soluble iron compounds present in the fermented mud. Leaves of Combretum aculeatum Vent have been subjected to a preliminary antibacterial screening against two Gram positive bacteria (Bacillus subtilis and Staphylococcus aureus), and two Grame

negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) the methanol and aqueous extracts showed high activity against the two gram-positive as well as gram -negative bacteria (Omer., *et al*. 2001).

Combretum species are widely used medicinal plants in Africa and Asia for many medicinal purposes. Several phytochemical investigations of the genus focus mainly on pentacyclic triterpenoids [1,2] and various polyphenols like flavonoids and stilbenoids [3]. In spite of all the earlier work done, there is still lack of data on the chemistry of some of the West African species like *C. aculeatum*, *C. glutinosum* and C. micranthum. Herein, we present a preliminary comparative profiling of triterpenoids and stilbenoids from the dichloromethane fractions of leaf and stem bark of *C. aculeatum, C. glutinosum* and *C. micranthum* using GC-MS. The terpenoids and stilbenoids were identified by comparing their mass spectrum to those of ursolic acid and combretastatin A4, respectively (Simon, 2003). Ursolic acid was identified in the leaf extracts of all the three species whereas combretastatin A4 was found in small amounts only in the bark extract of *C. glutinosum*. The chromatograms showed high variations between species and also between the two studied organs (leaf and bark). Triterpenes were mainly observed in the leaf extracts, while stilbenes were more concentrated in the bark extracts. Will contribute to the selection of highly active species for medicinal uses and to their authentication (Litaudon., *et al*. 2009). The result this *C. adenogonium* stem, *C. adenogonium* leaves, *C. adenogonium* roots, their IC_{50} are 1.03, 5.4, 7.2, µg/ml respectively all result in anti-oxidant in this plant parts is very high activity. This result agree with previous study was done by (Mariod., *et al*. 2006) reported that the antioxidant activity of the methanloic extracts of *C. hartmannianum* measured by DPPH free radical showed high reduction of 50%. Also all species under this study in the *C. adenogonium* cytotoxicity fixed all species toxic except *C. adenogonium* leaves. (Table1 – table 4)

Scientific name	Part used	IC_{50} ± SD
C. adenogonium ium. Steud. ExA. RICH	roots	> 100
	leaves	43.85 ± 0.85
	stem	> 100

Table 1: *IC_{50} of the methanol extracts of the selected Sudanese medicinal plants for cytotoxicity against Hela (cervical cancer) cell line proliferation.*

Scientific name	Ethyl acetate extract	Aqueous extract
	IC_{50} ± SD	IC_{50} ± SD
C. adenogonium (roots) ium. Steud. ExA. RICH	> 100	> 100
C. adenogonium (leaves) ium. Steud. ExA. RICH	43.85 ± 0.85	> 100

Table 2: *Screening of ethyl acetate and aqueous fractionations of Sudanese medicinal plants against Hela (cervical cancer) cell line proliferation.*

Scientific name	Part used	IC_{50} µg/ml
C. adenogonium ium. Steud. ExA. RICH	roots	> 200
	leaves	> 200
	stem	> 200

Table 3: *IC_{50} of the methanolic extracts of the selected Sudanese medicinal plants for cytotoxicity against MCF7 (breast cancer) cell line.*

Extract	Part used	Concentratio µg/mL	Inhibition % ± SD	IC_{50} (µg/mL)
C. adenogonium ium. Steud. ExA. RICH	roots	500	86.46091 ± 0.01	2.6
		250	86.45186 ± 0.02	
		125	84.58552 ± 0.04	
	leaves	500	88.70427 ± 0.01	
		250	82.45533 ± 0.06	In active
		125	75.88419 ± 0.02	
	stem	500	83.6829 ± 0.04	106.5
		250	82.18001 ± 0.07	
		125	54.75792 ± 0.15	

Table 4: *MTT cytotoxicity of 80% methanolic extract of selected Sudanese medicinal plants against Vero cell line.*

The Free Radical Scavenging Activity of Ic_{50} Dpph

The plants parts extracts (3) selected to be investigated for their IC_{50} against DPPH due to their inhibition activity at 50 µg/ml concentration. All the examined plants methanolic extracts which are *C. adenogonium* stem, *C. adenogonium* leaves, *C. adenogonium* roots, their IC_{50} are 1.03, 5.4, 7.2, µg/ml respectively. (Table5)

Test	Alkaloids	Sterols	Triter-penes	Flavo-noids	Saponins	Cumarins	Tannins	Anthraque-nones
C. adenogonium (roots)	-	-	+	+++	+	+	+++	-
C. adenogonium								
(leaves)	-	-	++	+++	+	+	+++	-
C. adenogonium								
(stem)	-	+++	++	+++	+++	+	+++	-

Table 5: *Result of phytochemical screening the selected plants methanolic extracts.*

Conclusion

In this study plant parts *C. adenogonium* were investigated for their anticancer and antioxidant activity to discover some new medicinal plants that can be used for treatement of cancer diseases. Anticancer activity of these plants was determined using. Best activity against Hela was shown by *C. adenogonium* leaves, with IC_{50}, 43.85µg/ml. The IC_{50} methanol extracts of the *C. adenogonium* roots (7.2 µg/ml), *C. adenogonium* leaves (5.4 µg/ml), *C. adenogonium* stem (1.03 µg/ml). The following extracts are alkaloids positive which adenogonium stem, high are in *C. adenogonium* roots, C. and moderate *C. adenogonium* leaves. There are flavonoids content was stem, *C. adenogonium* roost leaves and stem. There are saponins trace in C. adenogonium roots and leaves. The saponin content was high in *C. adenogonium* stem. There were cumarins trace in *C. adenogonium* roots, leaves and stem. There are tannins moderate in *C. adenogonium* roots, leaves and stem.

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